Allelopathic potential of root exudates of lucerne on annual ryegrass

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Summary Lucerne (*Medicago sativa* L.), also known as alfalfa in other countries, is a highly productive forage legume producing high quality feed for livestock. Yields deteriorate due to weed infestation. Herbicides are a key strategy for weed control, but the evolution of weeds resistant to herbicides requires consideration of alternative options such as allelopathic lucerne varieties for weed suppression. To evaluate the allelopathic prospects of lucerne, a laboratory based root exudates bioassay was conducted using the equal-compartment-agar method (ECAM). The allelopathic effects of different growth periods (0, 2, 4, 6, 8, 11, 15 days) and density (0, 5, 10, 15, 20, 30, 40, 50 pre-germinated seeds per beaker) of lucerne (var. Genesis) against annual ryegrass (*Lolium rigidum* Gaudin) were investigated. The root and shoot lengths of annual ryegrass were measured. Results indicated that lucerne growth for 4–8 days had the most inhibitive effect on the root growth of annual ryegrass. The root inhibition of annual ryegrass was increased with increasing density of lucerne. Growth period and density of lucerne however did not affect the shoot growth of annual ryegrass.

Keywords Allelopathy, growth period, density.

INTRODUCTION

Lucerne (*Medicago sativa* L.) is grown in different climates throughout the world and provides forage with high nutritive value for livestock (Al-Suhaibani 2010, Radović, Sokolović and Marković 2009). Its high level of digestible protein makes it a highly valued forage (Al-Suhaibani 2010). Weed infestations in crops and pastures reduce the quantity of yield, increase harvesting costs and compromise product quality. Weeds have been shown to decrease lucerne forage chemical composition as well as its palatability (Dimitrova and Marinov-Serafimov 2008).

Herbicide applications are a major tool for weed control in crops and pastures (Kidston, Ferguson and Scott 2010). However, weeds continue to evolve herbicide resistance threatening environmental sustainability and the viability of crop production (Peltzer *et al.* 2009). Australia has the challenge of limited synthesised herbicide options for effective weed control due to resistance and so alternative means of weed management need to be evaluated.

Allelopathy is a mechanism that refers to the direct or indirect detrimental or beneficial effects of one plant on the growth of another through production of chemical compounds (allelochemicals) released into the environment (Rice 1984). Root exudates represent one of the largest direct inputs of plant chemicals into the rhizosphere environment (Bertin, Yang and Weston 2003). Allelochemicals produced by plants have potential to reduce the effects of weeds in the field as biological herbicides and provide an alternative to commercial herbicides (Khanh *et al.* 2005).

Research has shown that extracts of different plant parts and residues of lucerne have allelopathic effects on the growth of lucerne itself (aotopathy) and several agricultural weeds (Abdulrahman and Habib 1989, Chon *et al.* 2002, Chung and Miller 1995). Furthermore, plant competitive ability can be increased by increasing plant population (Tollenaar *et al.* 1994), thereby lessening the negative effects from weeds (Berkowitz 1988). Further, growth duration affects the release of the allelochemicals in lucerne (Chung and Miller 1995a). Thus, crop growth period and density may have agronomic potential for non-chemical weed management. A preliminary experiment was conducted to determine the allelopathic potential of lucerne, through its density and early growth period, against annual ryegrass (*Lolium rigidum* Gaudin).

MATERIALS AND METHODS

Seed sterilisation and pre-germination Seeds of lucerne (var. Genesis) were surface sterilised by soaking the seeds in 2.0% sodium hypochlorite (NaOCl) solution for 15 minutes, then rinsed six times with sterilised distilled water. The surface sterilised seeds were transferred to a Petri dish with one sheet of Whatman No. 1 filter paper, moistened with 5 mL of sterilised distilled water, and sealed with Para film. Similarly, annual ryegrass seeds were placed to a

Petri dish. The seeds of lucerne and annual ryegrass were then incubated in a light/dark cycle of 12h/12h and a temperature cycle of 25°C /15°C in a controlled environment facility.

Growth period and density of lucerne A simple laboratory bioassay, the ECAM (equal-compartment-agar method) developed by Wu *et al.* (2000) was used to evaluate the allelopathic potential of lucerne to annual ryegrass. The treatments comprised seven different lucerne growth durations (0, 2, 4, 6, 8, 11 and 15 days). At each growth time, 30 pre-germinated and uniform lucerne seeds were sown on the aseptic agar surface of one-half of a glass beaker (600 mL) which was prefilled with 30 mL of 0.3% water agar. The beakers were sealed with Para film and kept in a controlled growth incubator (light/dark 12/12 h and 25°C/15°C). After growth of the lucerne seedlings for 0, 2, 4, 6, 8, 11, and 15 days, 10 pre-germinated seeds of ryegrass were transplanted on the other half (lucerne seeds were sown on the other half) of the agar surface. A piece of pre-autoclaved white cardboard was inserted across the centre and down the middle of the beaker with the lower edge of the cardboard kept 1 cm above the agar surface. The entire beaker was thereby divided into two equal compartments that were occupied separately by lucerne and ryegrass seedlings. Competition above the agar surface between lucerne and ryegrass was thus avoided by confining plants within their own compartments. The roots of lucerne freely entered the ryegrass compartment so that any allelochemicals produced and released by the lucerne seedlings could diffuse throughout the entire agar medium to influence ryegrass root growth. After ryegrass sowing, the beakers were again wrapped with Para film and placed back in the growth incubator for seven days. The growth of annual ryegrass alone was included as the control. The root and shoot lengths of the ryegrass seedlings were measured.

A second density experiment was conducted as per the above conditions. The lucerne densities used were 5, 10, 15, 20, 30, 40, and 50 seedlings per beaker.

Experimental design The experiments were set up using a randomised complete block design with four replications under controlled conditions. Data were subjected to analysis of variance using GenStat version 16.

RESULTS

Lucerne grown for 4 days significantly decreased ryegrass root length. Inhibition increased with extended lucerne growth period and reached a maximum after 8 days (Figure 1). Increasing the lucerne growth period beyond 8 days produced no further significant inhibition. The effect of lucerne density on ryegrass root length was also significant (Figure 2).

Increasing the density of lucerne seedlings significantly inhibited the root growth of annual ryegrass. The highest root length inhibition of annual ryegrass was found at the density of 50 lucerne seedlings per beaker (Figure 2). At this density, lucerne reduced annual ryegrass root growth by about 62%. Lucerne growth period and density did not affect shoot growth of annual ryegrass.

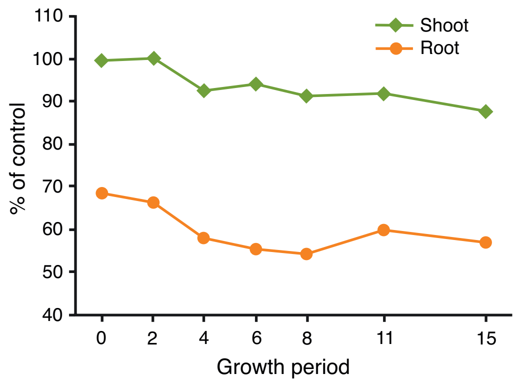

Figure 1. Effect of different growth periods of lucerne on root and shoot length of ryegrass. LSD (root) = 8.73, LSD (shoot) = 9.39, P <0.05.

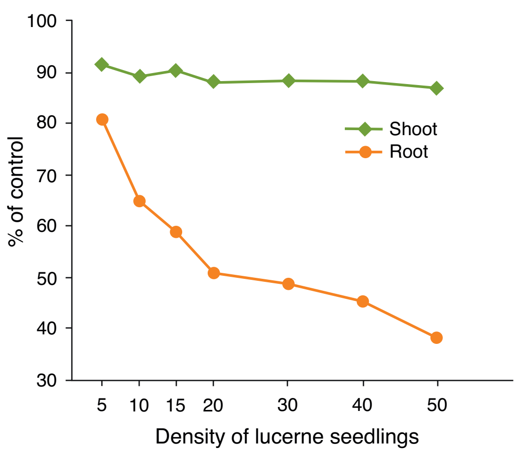

Figure 2. Effect of various densities of lucerne on root and shoot length of ryegrass. LSD (root) = 9.04, LSD (shoot) = 10.01, P <0.05.

DISCUSSION

The results indicated that the allelopathic activity of root exudates of lucerne was growth period-related and the maximum inhibition was caused by lucerne growth between 4 and 8 days. Huang *et al*. (2003) found a similar trend in wheat seedling phytotoxicity where the concentration of allelopathic compounds was greatest between 6–8 growing days after which the concentration declined. One explanation for this could be associated with the limited half life of these compounds in agar medium. We found that increasing the density of lucerne increased ryegrass root inhibition, most likely due to increased concentration of root exudates in the agar medium. Olofsdotter *et al.* (2002) noted that any compound can be toxic if applied at a high enough dose. Our results suggest that there is sufficient allelopathic potency to further explore the allelopathic prospects of lucerne against weeds.

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